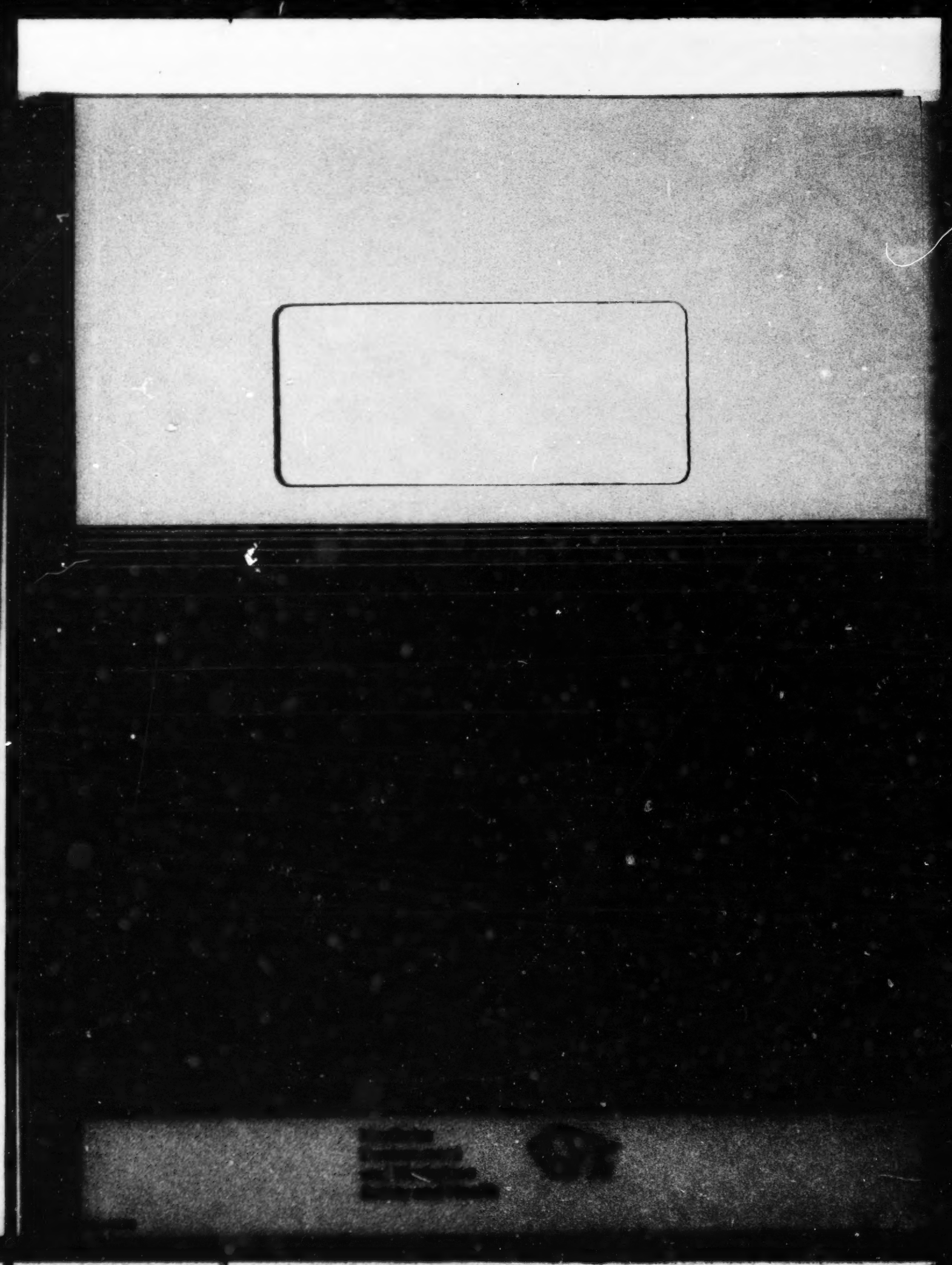

MONITORING SMELTER EMISSIONS USING SMALL MAMMALS AS INDICATORS

Emil Kucera, Ph.D.
Terrestrial Standards and Studies
Department of Environment and
Workplace Safety and Health
Province of Manitoba

Report 83-14
November 1983

Kucera, E. 1983
Monitoring smelter emissions using small mammals as indicators.
Manitoba Department of Environment and Workplace Safety and Health,
Environmental Management Division, Terrestrial Standards and Studies
Section. Report 83-14. 16 pp.

ABSTRACT

Free ranging mice and voles were trapped at experimental plots located from 4 to 185 km from the smelter. Blood samples were collected for hemoglobin determination, and tissues (hides and cleaned carcasses) were analyzed for six different metals.

Hemoglobin concentrations in blood of deer mice (Peromyscus maniculatus) were increased close to the smelter, probably as a result of the effect of sulphur dioxide emissions. The distance at which the hemoglobin concentrations drop to background levels was calculated to be 40 km from the stack.

Tissue concentrations of nickel (Ni) were below the 2.5 µg/g detection limit in all samples. Cobalt (Co) and zinc (Zn) also did not accumulate in the tissues in excess of normal background values. Arsenic (As), lead (Pb), and copper (Cu) were found in higher concentrations in animals from plots closer to the smelter than in animals from distant plots, and showed a statistically significant decrease with distance from the smelter. Metal concentrations in tissues of mice from distant plots did not differ significantly from background levels, i.e. those from the plot 185 km distant, which in turn were similar to levels found in animals from southern areas of Manitoba. Regression line fitting allowed an estimate of the distances at which the tissue concentrations of individual metals decrease to background levels. These were as follows: Pb, 23 km; As, 30 km; and Cu, 46 km.

The tissue concentrations of As and Pb are up to five times higher than background at plots near the smelter (about 1.5 times higher for Cu). Although no obvious effects on mice were discovered, it appears that voles (Clethrionomys gapperi) are adversely affected by the smelter emissions, because their population from 1978 through 1982 was low or nonexistent at plots up to 37 km from the smelter.

Table of Contents

	Page
Introduction	1
Materials and Methods	2
Sampling	2
Chemical Analysis	3
Data Evaluation	3
Results	5
Hemoglobin	5
Tissue Metals	6
House and Vole Populations	8
Discussion and Conclusions	9
Literature Cited	11

List of Tables

Page

Table 1. Sampling locations and years of small mammal collection..... 12

Table 2. Blood values of Peromyscus maniculatus at various distances from the Flin Flon smelter. Mean (range)..... 13

Table 3. Metal concentrations in tissues of Peromyscus maniculatus collected at various distances from the Flin Flon smelter; µg/g, mean (range)..... 14

Table 4. Correlations of tissue metal concentrations; t-values..... 15

Table 5. Catch of Peromyscus and Clethrionomys (animals/100 trap nights)..... 16

INTRODUCTION

A study of small mammals was initiated in 1977 to assist the Manitoba Clean Environment Commission in its deliberations leading to the setting of emission standards for northern base metal smelter operations. Preliminary results were presented to hearings regarding the Hudson Bay Mining and Smelting Co., Ltd. in Flin Flon (Kucera 1980) and Inco Ltd. in Thompson (Kucera 1981).

The study took a dual approach, looking at the animals

(a) as recipients of the emissions' impact; and

(b) as indicators of the area over which the impact of the emissions is felt.

The basic assumption is that an effect of a pollutant will be manifested by gradual physiological changes before clinical symptoms appear or death of an organism occurs. Mortality of individuals, or disappearance of populations are the ultimate, undisputable indications of adverse environmental conditions.

Although toxic effects on individuals will eventually be translated into changes at population level, both population size and causes of decline are difficult to assess and prove. In contrast, some physiological changes are easier to detect and quantify.

This report presents data on selected hematological values and on tissue concentrations of heavy metals for the deer mouse (Peromyscus maniculatus), and a comparison of relative numbers of deer mice and red-backed voles (Clethrionomys gapperi).

MATERIALS AND METHODS

Sampling

Locations of all sampling areas are shown in Table 1. Blood samples were obtained from 161 deer mice collected from 13 different areas during the summer months of 1977, 1979, and 1981. The locations changed slightly in 1979, therefore the results were pooled according to five distance zones as specified in the table. Tissues for heavy metal analysis were obtained from five locations within the above mentioned zones both in 1981 and 1982. Total sample for metal analysis comprised 128 deer mice.

Sherman live traps and Museum Special snap traps were used to catch the animals. Each live-trapped mouse was placed under light chloroform anesthesia, and a sample of blood was collected from the orbital sinus directly into a heparinized capillary tube. One drop of blood was used to determine the hemoglobin (Hb) concentration (g/100mL), then the capillary was centrifuged on a clinical centrifuge, packed cell volume (PCV) was recorded (percent), and the plasma protein (PP) content was measured with a Goldberg refractometer (percent). All these measurements were performed immediately after checking the traps at dawn and dusk, usually directly on site. Following blood sampling the mouse was killed by an overdose of the anesthetic. Hides and eviscerated carcasses were kept in separate vials in a freezer during the field trip, and subsequently freeze-dried for storage until chemical analysis. Samples were freeze-dried for 48-72 hours at 10-30 x 10^{-3} torr.

Chemical Analysis

All analyses were carried out by the provincial W. H. Ward Technical Services Laboratory. The samples were ashed, acid digested, and analyzed by atomic absorption spectrometry. Carcass samples were analyzed for Co and Pb, hide samples were analysed for Zn, Cu, Ni, and As. Because As analysis requires different digestion the hide samples had to be divided: in cases when there was not enough for both analyses preference was given to Zn, Cu and Ni, and some As analyses were made of samples that were pooled, i.e. included material from more than one animal.

The detection limits, based on an average .75 g sample, were as follows:

Ni, Co, Pb	2.5 µg/g.
Zn, Cu	.7 µg/g.
As	.2 µg/g.

Data Evaluation

Mean values were calculated for both hematological values and metal concentrations for animals in each of the five distance zones. Analyses of variance and covariance, and multiple range tests were employed to compare the means. Relationships of hemoglobin in blood, and metal in tissue concentrations to distance were expressed by an appropriate regression. Finally, two separate regression lines were calculated: One through the significantly elevated values encountered near the smelter, the other through values not significantly different from background (as shown by the multiple range test).

The distance corresponding to the intersection of these two lines is postulated to be the distance to which the particular component of the emissions is carried in sufficient quantities to elicit a detectable physiological response in the animals.

Standard procedures and programs available in the VS-APL system at Manitoba Data Services were used for the statistical analysis.

RESULTS

Regression

For area comparisons the mean values were adjusted for both plasma protein levels and for presence or absence of parasitic larvae, using analysis of covariance. A number of mice in Flin Flon area were infested with warble-type larvae under the skin (species not identified at the time of this writing), and the presence of larvae significantly depressed hemoglobin (Hb) levels ($t=4.65$, $p<.01$). Sealander (1961) reported such effects for botfly infestation. Plasma protein levels were also significantly correlated to Hb concentration ($t=2.97$, $p<.01$), suggesting that some of the Hb readings could have been artificially high owing to dehydration.

Table 2 shows the comparison of adjusted mean values of Hb, PCV, and mean corpuscular hemoglobin concentration (MCHC) for the five distance zones. Although all values are within the normal range reported in the literature (Sealander 1962, 1964; Sawin 1970; Dieterich 1973), both Hb and MCHC values are highest near the smelter and decrease with distance from it. The decrease in Hb is statistically significant and can be expressed by a regression:

$$Hb = 11.82 + .56PP - 3.54W - .012Dist$$

$$(R^2 = .35,\ F = 28.43,\ p<.01)$$

where Hb is hemoglobin concentration (g/100mL), PP is plasma protein concentration (percent), W is warble infestation (1=present, 0=absent), and Dist is distance (km).

Although linear, this relationship can not be carried ad infinitum; since the multiple range test showed no significant difference between zones 1 to 4 and 4 to 5 (Table 2), separate regressions were derived for the two groups. These were: For zones 1-4

$$Hb = 11.051 + .708PP - 3.626W - .016Dist$$

$$R^2 = .37,\ F = 21.62,\ p<.01;$$

for zones 4-5 $Hb = 12.204 + .493PP - 4.185W - .012Dist$

$$R^2 = .29,\ F = 8.60,\ p<.01.$$

To find the distance at which the Hb value drops to the background level the overall mean values are substituted for W and PP, and the two regressions combine into a simple equation with one unknown (Dist).

$$11.051+.708x5.785-3.626x.124-.016Dist=11.051+.708x5.785-4.185x.124-.012Dist$$

$$Dist=40$$

As discussed by Kucera (1981), and supported by the research of Greenwald (1954), Mylrea and Abbrecht (1970), Sawin (1970, and in particular Novakova and Hanzl (1968) and Singh (1976), the most likely agent causing the rise in Hb concentration near the smelter is the sulphur dioxide (SO_2) emitted from the stack. On the basis of these data it appears that SO^2 emissions are carried as far as 40 km in sufficient quantities to cause a mild respiratory stress to deer mice.

Tissue Metals

The results of metal analyses are presented in Table 3, together with results of multiple range tests comparing the mean values for animals sampled at different distances from the smelter. Except for Zn (lower in 1982) there were no significant differences in

metal concentrations between 1981 and 1982 samples, therefore all data were pooled for both years. Nickel was not found in detectable amounts in any of the samples and Cc and Zn showed no consistent correlation with distance from the smelter. However, distinctly higher concentrations of As, Cu and Pb were found in tissues of mice caught close to the smelter compared to those collected farther away. Metal concentrations in animals from zone 5 were not significantly different from those found in Southern Manitoba, not subjected to smelter emissions (Environmental Management, data on file).

The relationship of As concentration to distance from the smelter is best described by a simple regression of the form

$$As = 2.148 - .49 \ln Dist$$

$$r^2 = .78,\ t = 9.72,\ p<.01,$$

where As is As concentration in μg/g, and Dist is distance (km). Using the same reasoning and procedure as described above for hemoglobin, separate regressions can be derived for zones 1-2 and 3-5, and then combined into an equation which is solved for the distance:

$$.456 - .045 \ln Dist = 2.385 - .610 \ln Dist$$

$$Distance = 30 \text{ km}$$

Simple regressions for the other metals, although highly statistically significant, produced rather low coefficients of determination (r^2 = .23 for Cu, .25 for Pb). However, the tissue concentrations of the studied metals were mostly significantly correlated (Table 4), and using multiple regressions the R^2 coefficients were much higher: .34 for Cu, .50 for Pb (F=21.66 and 30.12 respectively, p<.01 in both cases). The best fit regressions were as follows:

$$Cu = 7.659 + .131Pb + .038Zn - .678 \ln Dist$$

$$\ln Pb = 1.914 + .373Co - .006Zn + .043Cu - .204 \ln Dist$$

Distances at which the metal concentrations would decrease to the background levels were calculated in the same way as for Hb or As, resulting in

46 km for Cu, and

23 km for Pb.

Mouse and Vole Populations

A summary of catches of both deer mice and red-backed voles is shown in Table 5. The vole-to-mouse ratio is also given. The red-backed vole is usually the most common small mammal of the boreal forest, but its abundance was low in the three study zones closest to the smelter. Although it would be difficult to prove, it seems to be a reasonable assumption that the smelter emissions are implicated. Some support for the hypothesis is provided by studies from the area near Inco Ltd. smelter in Thompson (Kucera 1981), and also by preliminary results reported by West et al. (1981). Voles were scarce near the Thompson smelter as well. West compared populations of small mammals on sludge-fertilized, heavy metal rich soils, with controls, and found herbivores such as voles less numerous on the experimental plots. Granivorous Peromyscus was much less affected. Voles also had higher concentrations of metals in their tissues.

DISCUSSION AND CONCLUSIONS

The results can be evaluated according to the criteria stated in the Introduction, i.e. firstly, regarding the effects of emissions on the animals themselves, and secondly, regarding the function of the animals as indicators of the emissions' distribution.

The vole population seems to be to some extent adversely affected by the emissions. There is no apparent effect on the mouse population. Given the normal range of Hb concentrations, the slight rise in values near the smelter cannot be considered harmful. There is no apparent reason to attribute the vole decline to the sulphur dioxide effects, or to consider the voles more susceptible than mice in that respect. Metals, on the other hand, have a greater apparent potential to cause harmful effects, owing to their known toxicity. Voles could be more susceptible than mice because of their different food habits, being largely herbivorous, in contrast to the mainly granivorous mice. The voles would ingest a greater amount of metals, both from the plant material and from the deposition on the plant surface. Seeds would contain a lower amount of metals. It is also suggestive that the area with lowest vole population corresponds well to the area over which the animals had elevated concentrations of the two most toxic metals, As and Pb.

The small mammals proved useful as indicators of the area under the impact of the emissions, at least in the SE quadrant where study plots were located. Based on concentration in the tissues, distances from 23 to 46 km were calculated for significant dispersion

of different metals. The differences may be due to the chemical form and behaviour of the emitted particulate matter. It is not possible to assess the extent to which the tissue concentrations reflect the current emission levels vs. the accumulated past deposition. However, it seems reasonable to expect that the freshly deposited compounds would exert a greater influence on the animal than material that has been altered over a period of time by weathering or action of biological agents. Lower tissue concentrations of Zn observed in 1982 when much less Zn had been emitted from the smelter, (Hudson Bay Mining and Smelting, 1982) tend to support such reasoning. Although some accumulation of metals in the environment would inevitably occur at any level of emissions, organisms can metabolize small amounts of toxics much easier than large amounts. Biological considerations definitely argue for the lowest possible emission levels.

Literature Cited

Greenwald, I. 1954. Effects of inhalation of low concentrations of sulphur dioxide upon man and other mammals. A.M.A. Arch. Ind. Hyd. Occ. Med. 10: 455 - 475.

Hudson Bay Mining and Smelting Co. Ltd. 1982. Manitoba Government stack emission compliance test. Letter to Environmental Management dated Nov. 18, 1982.

Kucera, E. 1980. Emissions distribution reflected in wild rodent tissues. Submission to Manitoba Clean Environment Commission hearing in connection with H.B.M.&S. Co. Ltd. smelter operation at Flin Flon.

_____. 1981. Effects of emissions on small rodents near Thompson smelter. Submission to the Clean Environment Commission Hearings in connection with INCO Ltd. smelter operation at Thompson, Manitoba.

Mylrea, K.C., and P.H. Abbrecht. 1970. Hematologic responses of mice subjected to continuous hypoxia. Amer. J. Physiol., 218: 1145 - 1149.

Novakova, E., and R. Hanzl. 1968. Erythrogramme du lievre commun (Lepus europaeus Pall.) dans les regions cultivees. Lynx 9: 38 - 60. (In Czech, French Summary).

Sawin, C. F. 1970. Hematology of sea level and high altitude native Sonoran deer mice. Amer. J. Physiol. 218: 1701 - 1706.

Sealander, J. A. 1961. Hemological values in deer mice in relation to botfly infection. J. Mammal 42(1): 57 - 60.

_____. 1962. Seasonal changes in blood values of deer mice and other small mammals. Ecology 43: 107 - 119.

_____. 1964. The influence of body size, season, sex, age and other factors upon some blood parameters in small mammals. J. Mammal. 45: 598 - 616.

Singh, J. 1976. Effects of sulphur dioxide exposure on the postnatal development and hematological studies in mice. Inst. Environ. Sci. Proc. 22: 147 - 151.

West, S. D., R. D. Taber, and D. A. Anderson. 1981. Wildlife in sludge-treated plantations. Pages 115 - 122 in C. S. Bledsoe (ed.) Municipal sludge application to Pacific northern forest lands. Inst. For. Resour., Univ. Washington, No. 41. Seattle.

Table 1. Sampling locations and years of small mammal collection

Zone	1			2				3			4		5
Distance from smelter (km)	4	5	6	9	11	15	16	36	37	38	61	68	185
Azimuth (degrees)	135	100	158	126	135	172	110	166	159	131	151	82	109
Blood sampling (year)	81	78	78	78	81	78	78	78	81	78	81	78	77,79,81
Tissue collection (year)	81,82				81,82				81,82		81,82		81,82

Table 2. Blood values of Peromyscus maniculatus at various distances from the Flin Flon smelter[1]. Mean (range).

Zone	1	2	3	4	5
Distance (km)	4 - 6	9 - 16	36 - 38	61 - 68	185
n	19	53	33	12	44
Hemoglobin (g/100 mL)	14.59[a]	14.46[a]	14.28[a]	13.49[a,b]	12.41[b]
	(7.6 - 19.9)	(6.3 - 17.8)	(7.8 - 18.5)	(6.8 - 16.7)	(7.2 - 15.0)
Packed cell volume (%)	44.3[a]	45.2[a]	45.4[a]	45.3[a]	40.7[b]
	(24 - 58)	(26 - 62)	(25 - 59)	(34 - 54)	(23 - 50)
MCHC (100xHb ÷ PCV)	33.1[a]	32.0[a,b]	30.5[b,c]	29.7[c]	30.7[b,c]
	(30 - 44)	(23 - 35)	(28 - 36)	(20 - 35)	(26 - 35)

[1]Means adjusted for plasma proteins and parasite infestation. Within each row, means with different superscripts are significantly different ($p<.05$, Duncan's multiple range test).

Table 3. Metal concentrations in tissues of Peromyscus maniculatus collected at various distances from the Flin Flon smelter; µg/g, mean (range)[1].

Zone		1	2	3	4	5	
Distance		4	11	37	61	185	Total
	n	7	10	8	4	5	34
Hide	As	1.54[a]	0.92[b]	0.26[c]	0.37[c]	0.20[c]	
		(1.39 - 2.01)	(0.58 - 1.34)	(0.13 - 0.45)	(0.09 - 0.99)	(0.07 - 0.45)	
	n	14	44	28	9	33	128
Hide	Zn	132.5[a]	112.8[b]	140.7[a]	117.0[b]	101.7[b]	
		(78 - 182)	(78 - 182)	(94 - 187)	(83 - 167)	(78 - 124)	
	Cu	14.63[a]	11.43[b]	9.97[b,c]	9.00[c]	9.39[c]	
		(9.3 - 26.6)	(8.1 - 19.1)	(5.4 - 14.6)	(6.3 - 11.4)	(1.0 - 13.5)	
	Ni	<2.5	<2.5	<2.5	<2.5	<2.5	
Carcass	Co	2.21[a]	2.09[a,b]	1.81[b]	1.81[b]	2.25[a]	
		(1.2 - 3.0)	(1.2 - 4.0)	(1.0 - 3.2)	(1.4 - 2.4)	(1.2 - 3.2)	
	Pb	13.81[a]	8.04[b]	3.12[c]	3.20[c]	5.30[c]	
		(8.1 - 33.3)	(2.7 - 30.4)	(2.5 - 5.3)	(2.5 - 4.0)	(2.9 - 11.0)	

[1]Within each row, means with different superscripts are significantly different ($p < .05$, Duncan's multiple range test).

Table 4. Correlations of tissue metal concentrations; t-values.

	Pb	Zn	Co
Cu	4.37**	4.90**	0.62
Pb		0.37	3.41**
Zn			1.21

**p<.01

Table 5. Catch of Peromyscus and Clethrionomys (animals/100 trap nights).

Zone	1	2	3	4	5
Clethrionomys					
1978	0	0	0.01	0.01	0.91
1981	0	0.27	0.94	1.08	4.57
1982	0	0.86	0	0	—
Peromyscus					
1978	0.02	0.02	0.03	0.01	3.77
1981	1.08	3.76	3.09	0.67	4.57
1982	2.86	8.86	0.71	0.57	4.57
Clethrionomys to Peromyscus ratio					
1978	0	0	0.33	1.00	0.24
1981	0	0.07	0.30	1.6	1.00
1982	0	0.10	0	0	—
Overall C.: P. ratio	0	0.06	0.21	0.87	0.62

POOR

QUALITY

ORIGINAL